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## COMPARATIVE BIOLOGICAL IN VITRO STUDIES ON PREDICTION OF EYE IRRITATION

The following material was presented by Gibraltar Biological Laboratories, Inc. on December 15, 1989 at a meeting of the SOCIETY OF COMPARATIVE OPHTHALMOLOGY, at Newark, New Jersey together with a set of principles for evaluation. The paper discusses the use of living biological systems opposed to inanimate chemical systems for predicting injury in animal and man.

### SUMMARY AND PRINCIPLES

Alternative safety assays are chemical or ascending biological such as (a) unicellular (bacteria, protozoa) (b) multicellular (cell culture) (c) diploblastic or (d) true metazoan (chick embryo triploblastic). The chemical tests and the biological tests may or may not predict eye irritation in the rabbit. They do not predict reversibility or rinse mitigation. Materials are more cytotoxic in liquid state elution assays than in solid state agar overlay assays. High titer cell culture reactivity and CAM hemorrhage/necrosis may predict corneal opacity. Broad spectrum antimicrobial activity also assists in predicting irritation. Formulations showing the same endpoint in cell culture may or may not score equally in the rabbit eye. Practically all substances are toxic in vitro, being dose-concentration-and time-dependent and interpretation requires side-by-side analysis, or a solid in vivo data bank. Use of in vitro tests for prediction will vary according to formulation composition. These tests can be useful only when endpoints have been correlated to doses and concentrations of negative controls and to doses and concentrations of controls known to be irritating in vivo. Eye irritation is a composite of numerous subcomponents ranging from membrane effects and lysis to secretion, coagulation and autonomic effects; different in vitro endpoints will speak to different portions of this continuum. New substances or formulations can be fruitfully pre-screened for presumptive effects in vitro. However, without final in vivo safety testing (such as the Draize), they should be administered with caution and with informed consent to both pre-market volunteers, who are replacing animals, or to the end-user of the product or drug. The issue of liability or injury at the patient or consumer level, whether by use or misuse of an article, should be taken into consideration when we explore how far an alternative assay (chemical or ascending biological) has departed from practical application.

TABLE 7  
PREDICTION OF RINSE MITIGATION

PRODUCT	CELL CULTURE	CAM	CORNEAL OPACITY RABBIT EYE	
			NO RINSE	RINSE*
EXPERIMENTAL HAIR FORMULA PH = 6.7	+ ] EQUIVALENT	+ ] EQUIVALENT	↔ 3/3 (21)	⊙ 3/3 (27) ↓
CONTROL HAIR FORMULA PH = 6.2			↔ 2/3 (18)	↔ 2/3 (16)

\* 20 ML TAP WATER AFTER 20 SECONDS

↔ Reversible  
⊙ = DRAIZE SCORE  
↓ IRREVERS.

CONCLUSION

1. EXPERIMENTAL FORMULA WORSE AFTER RINSING THAN CONTROL FORMULA (VERIFIED ON REPEAT EXPERIMENTS). RINSING TENDED TO INCREASE THE INCIDENCE OF OPACITY AND CAUSE IRREVERSIBILITY.
2. ALTERNATIVE TESTS SHOWED BOTH PRODUCTS THE SAME.
3. ELIMINATION OF THE DRAIZE RINSE TESTS WOULD HAVE BEEN MISLEADING.
4. ALTERNATIVE TESTS MUST ADDRESS THE PROBLEM OF RINSE MITIGATION.
5. PRODUCT WAS SUCCESSFULLY REFORMULATED BASED UPON RABBIT DATA.

TABLE 3  
COMPARISON OF EYE HAZARD AND DISINFECTANT  
WITH ADULT AND BABY HAIR FORMULAS

DILU- TION	CELL CULTURE DILUTION (2 HR.)			CELL CULTURE AGAR DIFFUSION			CAM*		
	HAIR			HAIR			HAIR		
	DISF	ADULT	BABY	DISF	ADULT	BABY	DISF	ADULT	BABY
1:2	+	+	+	+	+	+	+	+	+
1:4	+	+	+	+	+	+	+	+	+
1:8	+	+	+	+	+	+	+	+	+
1:16	+	+	+	+	+	+	+	+	+
1:32	+	+	+	+	+	+	+	+	+
1:64	+	+	+	+	+	+	+	+	+
1:128	+	+	+	+	+	+	+	+	+
1:256	+	+	+	+	+	+	+	+	+
1:512	+	+	+	+	+	+	+	+	+
1:1024	+	+	+	+	+	+	+	+	+
1:2048	+	+	+	+	+	+	+	+	+
1:4086	+	+	+	+	+	+	+	+	+

NO ENDPOINT      NO ENDPOINT + = TOXIC      0 = NEGATIVE

CONCLUSION

1. ALL THREE ALTERNATIVE ASSAYS RANKED THE EYE HAZARD DISINFECTANT AS SIGNIFICANTLY MOST TOXIC.
2. NEITHER THE CELL CULTURE NOR THE CAM PREDICTED CORNEAL OPACITY FOR ADULT SHAMPOO.
3. HIGH TITER ENDPOINTS ON ALL THREE TESTS MAY PREDICT CORNEAL OPACITY FOR A KNOWN EYE HAZARD.
- \* COMPOSITE OF READING ON NECROSIS, HEMORRHAGE, VASODILATION (VARIABLE PARAMETERS).

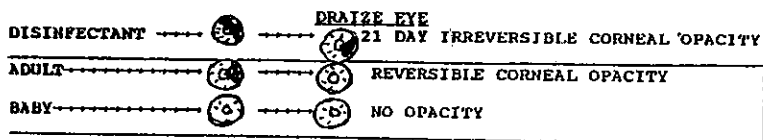


TABLE 4  
COMPARISON OF CELL CULTURE, CAM AND  
GHI. MICROBIAL ASSAY ON PREDICTING  
IRREVERSIBLE CORNEAL OPACITY

PRODUCT	CELL CULTURE <sup>1</sup>	CAM <sup>2</sup>	MICROBIAL <sup>3</sup> DIFFUSION	IRREVERSIBLE CORNEAL OPACITY
BABY SHAMPOO	+	0	0	0 ☉
ADULT SHAMPOO	+	0	0	0 ☉
EXPERIMENTAL SHAMPOO	+	+	0	0 ☉
BUBBLE BATH	+	0	0	0 ☉
DISINFECTANT	+	+	+	+

CONCLUSION

HIGH TITER ON THE DILUTION ASSAYS AND BROAD SPECTRUM ANTIMICROBIAL ACTIVITY PREDICTED IRREVERSIBLE CORNEAL CAPACITY.

- 1 + = TOXICITY AT A DILUTION OF > 1:32
- 2 + = TOXICITY AT A DILUTION OF > 1:32
- 3 ZONE INHIBITION 3/3 ORGANISMS

TABLE 5  
PHYLOGENETIC APPROACH BASED  
ON TOXICITY OF  
SODIUM LAURYL SULFATE

SYSTEM	μ TO PRODUCE EFFECT
RABBIT CORNEAL OPACITY	10.0
RABBIT SKIN IRRITATION (>2.0)	10.0
DRAIZE EYE SCORE (>15)	5.0
GRAM NEGATIVE BACTERIA (INHIBITION)	5.0
RABBIT FLUORESCHEIN EYE AT 2 HOURS	5.0
CHICK EMBRYO	2.5
RABBIT EYE DISCHARGE AT 2 HOURS	1.0
GUINEA PIG IMMERSION (SKIN IRRITATION)	0.125
CELL CULTURE (LYSIS GRANULATION)	0.025
HERPES/INFLUENZA VIRUS (INACTIVATION)	0.0125 (125 PPM)
GRAM POSITIVE BACTERIA (INHIBITION)	0.0020 (20 PPM)

CONCLUSION

1. MICROBIAL AND CELL CULTURE SYSTEMS ARE 10 TO 10,000 TIMES MORE SENSITIVE THAN IN VIVO SYSTEMS.
2. ~~CELL CULTURES ARE THE MOST SENSITIVE MAMMALIAN SYSTEM~~
3. ~~EYE DISCHARGE AND FLUORESCHEIN STAINING OF CORNEAL EPITHELIUM ARE THE MOST SENSITIVE COMPONENTS OF THE RABBIT EYE IRRITATION TEST AND ARE MORE LIKELY TO BE PREDICTED BY CELL CULTURE THAN BY CAM BUT CAN BE PREDICTED BY BOTH.~~

TABLE 6  
POTENTIAL RANGE OF  
PREDICTABLE RESPONSES

PARAMETER		CELL CULTURE	CAM (DROPPED MEMBRANE)
DISCHARGE		+	+
CONJUNCTIVITIS		+	+
FLUORESCHEIN STAINING OF CORNEAL EPITHELIUM		+	+
IRITIS		?	?
CORNEAL OPACITY		?	?
REVERSIBILITY		0	0
RINSE MITIGATION		0	POSSIBLE
ENDPOINT	EFFECT	LYSIS, DYE UPTAKE	HEMORRHAGE
	STANDARDIZED		NO
			NECROSIS
			VASODILATION

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TABLE 1  
COMPARATIVE SIDE-BY-SIDE RESULTS  
CELL CULTURE COMPARED TO DRAIZE

DILUTION	MEM DILUTION (2 HOURS)		AGAR OVERLAY		
	ADULT GREASELESS HAIRTONIC (EXPT)	ADULT GREASELESS HAIRTONIC (CONTROL) (Marketed)	EXPT	CONT.	DRAIZE
1:2	+	+	+	+	
1:4	+	+	+	+	
1:8	+	+	[+] = [±]		
1:16	[+]	= [±]	0	0	
1:32	0	0	0	0	
1:64	0	0	0	0	
1:128	0	0	0	0	
1:256	0	0	0	0	
TOXIC ENDPOINT	[1-16]	[1-16]	[1-8]	[1-8]	
DRAIZE WITHOUT RINSE	SCORE = 21 3/3 OPACITY	SCORE = 18.0 HIGHEST 2/3 OPACITY			



+ = CYTOTOXIC      0 = NEGATIVE



CONCLUSION

- BOTH CELL CULTURE ASSAYS SUCCESSFULLY SHOWED EQUIVALENCE OF RESPONSE IN THE DRAIZE RABBIT EYE TEST.
- THE AGAR OVERLAY WAS LESS SENSITIVE THAN THE DILUTION ASSAY.
- ELIMINATION OF THE DRAIZE EYE TEST WOULD HAVE BEEN BENEFICIAL.
- THE EXPERIMENTAL WAS NO WORSE THAN THE MARKETED EQUIVALENT.

TABLE 2  
BABY PRODUCTS

DILUTION	MEM DILUTION (2 HOURS)		AGAR OVERLAY	
	MARKETED SHAMPOO	EXPERIMENTAL BATH	MARKETED SHAMPOO	EXPERIMENT BATH
1:2	+	+	+	+
1:4	+	+	+	+
1:8	+	+	+	+
1:16	+	+	+	+
1:32	+	+	[+] = [±]	
1:64	+	+	0	0
1:128	[+]	+	0	0
1:256	0	= [±]	0	0
1:512	0	0	0	0
TOXIC ENDPOINT	[1-128]	[1-256]	[1-32]	[1-32]
DRAIZE WITHOUT RINSE	SHAMPOO SCORE = 7.7  NON-IRRITANT	BATH SCORE = 19.3  IRRITANT FLUORESCEN STAINING		

+ = CYTOTOXIC      0 = NEGATIVE

CONCLUSION

- THE CELL CULTURE ASSAYS WERE SIMILAR AGAIN.
- THIS EQUIVALENCE DID NOT PREDICT EYE IRRITATION
  - CONJUNCTIVITIS
  - CORNEAL DAMAGE - FLUORESCEN STAINING
- ELIMINATION OF THE DRAIZE TEST WOULD HAVE BEEN HARMFUL.
- THE EXPERIMENTAL WAS WORSE THAN THE MARKETED EQUIVALENT.

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